
Genetic methods to detect vertebrate herbivory on Tiehm's buckwheat (*Eriogonum tiehmi*): FINAL REPORT

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Executive Summary:

Environmental DNA (eDNA) was analyzed from damaged roots of Tiehm's buckwheat (*Eriogonum tiehmi*), undamaged control root samples taken from undamaged plants, soil tailings adjacent to damaged buckwheat plants, control soil collected from undamaged plants, and rodent scat found near damaged plants. The analysis revealed genetic signatures of a rodent species belonging to tribe Marmotini, a taxon that includes antelope ground squirrels (*Ammospermophilus*), prairie dogs (*Cynomys*), marmots (*Marmota*), Chinese rock squirrels (*Sciurotamias*), ground squirrels and rock squirrels (*Spermophilus*), and chipmunks (*Tamias*).

The genetic signatures range from 96.96 to 99.75% match ($e=0.0$) with the banked DNA sequence for Harris' antelope ground squirrel (*Ammospermophilus harrisi*) in the GenBank database maintained by the National Institutes of Health. The e-value is the number of expected DNA sequences of similar match quality that could be found just by chance. The closer e is to zero, the better the quality of the match. This does not mean that the DNA came from *A. harrisi*. The DNA most likely originated from the locally abundant white-tailed antelope ground squirrel (*A. leucurus*), which does not have a 12S DNA barcode banked in the GenBank or BOLD databases. These two antelope ground squirrels are expected to have extremely similar DNA sequences because they belong to the same genus.

These same *Ammospermophilus* genetic signatures were also found in soil samples and in the rodent scat (pellets) found within Tiehm's buckwheat subpopulation 2. The rodent pellets were also tested for plant eDNA, and found to contain DNA with a 100% match for the buckwheat genus, *Eriogonum*. A DNA barcode for Tiehm's buckwheat does not yet exist in GenBank or BOLD, which means that the nearest genetic match is *Eriogonum crosbyae* with which it has a 100% sequence match ($e=2 \times 10^{-65}$). No sciurid (squirrel family) eDNA was found in the control samples. These data strongly support the hypothesis that a diurnal rodent species in the genus

Ammospermophilus was responsible for damage to the Tiehm's buckwheat population at Rhyolite Ridge in the late summer or early fall of 2020.

Cervid (deer family) eDNA was present in five samples, and human DNA from sampling contamination was present in two samples.

Background: Damage to Tiehm's buckwheat

Tiehm's buckwheat (*Eriogonum tiehmii*) is a rare plant found on approximately 21 acres in the Silver Peak Range of Esmeralda County, Nevada (Reveal, 1985). On September 8, 2020 researchers from the Leger Lab at the University of Reno observed that an estimated 25-50% of plants distributed among six subpopulations had been damaged, cut, or dug up (McClinton, 2020). The damage was later noticed by biologists from other agencies who reported it to the media along with accusations of human malfeasance (Bahouth, 2020). Initial reports indicated that the damage was due to rodents (McClinton, 2020), but concern remained that humans may have been responsible. The work described below was undertaken to investigate potential causes of the damage.

Background: Environmental DNA

Environmental DNA (eDNA) is trace DNA found in soil, water, food items, or other substrates with which an organism has interacted. eDNA samples contain the DNA traces from multiple community members in the environment of interest. eDNA from residual saliva has been used to identify predator DNA from bites on clay models (Rößler *et al.* 2020), brown bear DNA left on salmon carcasses (Wheat *et al.* 2016), macaw DNA from fruits (Monge *et al.* 2020), and aye-aye DNA from feeding traces on trees (Aylward *et al.* 2018). These studies show that eDNA from residual saliva is a reliable way to detect when vertebrates have fed or attempted to feed on an item. Therefore, an eDNA analysis of damaged Tiehm's buckwheat roots could determine whether a vertebrate herbivore has left saliva on parts of plants still remaining in the field. Many rodent herbivores disturb the soil when they make burrows or shelters, which means that an eDNA analysis of disturbed soil near damaged plants may also reveal the genetic signature left behind by rodent herbivores.

Please note that Next Generation Sequencing (NGS) methods are extraordinarily sensitive, which means that human DNA contamination in the field-collected samples will be almost unavoidable.

Methods

Sample collection. On October 2, 2020 we obtained eDNA from three sources for this study: (1) depredated and control Tiehm's buckwheat plants at the site of root depredation, (2) soil from burrow tailings located directly next to depredated plants with soil from unaffected plants as a control, and (3) rodent scat found near depredated plants on the soil surface (Table 1, Figure A Appendix). Fifteen to twenty root samples were collected at Tiehm's buckwheat subpopulations 1, 2, 4, and 6. The root samples were combined into 5 pooled subsamples for subpopulations 1 and 6, and six pooled subsamples for subpopulations 2 and 4. Three intact plants were excavated

from subpopulation 1 and subpopulation 6a, for a total of six control plants from which we expected little to no evidence of herbivore eDNA. The number of control plants was minimized to prevent further damage to the subpopulations.

In a separate analysis, fifteen to twenty samples of burrow tailings were collected at Tiehm's buckwheat subpopulations 1, 2, 4, and 6. These samples were pooled to produce six separate samples for analysis from each subpopulation. Four control soil samples that consisted of soil collected within a 30 cm radius of untouched plants were collected from subpopulations 1, 2, 4, and 6.

Rodent scat was found at subpopulation 2, where approximately 12 individual pellets were found near damaged plants. This pooled sample of pellets was divided into two subsamples to allow for an examination of eDNA from vertebrate herbivores and a separate analysis of plants in the diet.

Table 1. Five types of samples were collected for this study of environmental DNA (eDNA).

Sample type	Rationale
1. Chewed, damaged, and depredated roots	eDNA from herbivory should be present and might allow identification of a potential herbivore. The <i>12S</i> mitochondrial rRNA barcode was used to sequence any vertebrate eDNA found on root samples.
2. Untouched roots from intact <i>E. tiehmii</i> plants	Any eDNA present on intact plants should be background eDNA inherent to the site, which allows these samples to serve as controls. The <i>12S</i> mitochondrial rRNA barcode was used to sequence any vertebrate eDNA found on control root samples.
3. Soil taken from burrow tailings located within 30 cm of damaged <i>E. tiehmii</i> plants	eDNA in the soil tailings should be closely associated with the herbivore that dug the holes. The <i>12S</i> mitochondrial rRNA barcode was used to sequence any vertebrate eDNA found in soil samples.
4. Soil taken from within a 30 cm radius of untouched <i>E. tiehmii</i> plants	Any eDNA present in untouched soil should be background eDNA inherent to the site, which allows these samples to serve as controls. The <i>12S</i> mitochondrial rRNA barcode was used to sequence any vertebrate eDNA found in control soil samples.
5. Rodent scat in the form of pellets found within subpopulation 2 of Tiehm's buckwheat	<p>Pellets can be tested for the presence of vertebrate eDNA, which would indicate the taxon from which they originated. The <i>12S</i> mitochondrial rRNA barcode was used to sequence vertebrate eDNA in a sample of six pellets.</p> <p>Pellets can also be tested for the presence of plant eDNA, which would indicate whether or not DNA from <i>Eriogonum</i> had been ingested by the animal. The <i>trnL</i> barcode is specific to plants and was used to sequence plant eDNA in a sample of six pellets.</p>

	<i>E. tiehmii</i> does not yet have any DNA barcodes in the BLAST database that is coordinated by the National Institutes of Health (NIH), which means that any <i>Eriogonum</i> DNA found in our samples can only be identified to genus.
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Sequencing. Samples were stored on ice in a portable cooler in the field and during shipping. Because our goal was to determine whether herbivore eDNA was present or absent in root and soil samples, we pooled root samples from 1-3 plants, and 2-4 soil subsamples per site (Appendix. Table A). Illumina Next Generation Sequencing (NGS) was performed by Jonah Ventures (Boulder, CO). Sequence data was matched to in-house libraries hosted by Jonah Ventures and to sequences located in GenBank by using the Basic Local Alignment Search Tool (BLAST) web tool hosted by the NIH (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) on 11/10/20. The BOLD Systems Public Data Portal was checked on 11/12/20 for the existence of banked sequences for *Ammospermophilus leucurus* and *Eriogonum tiehmii*.

Results & Discussion

Root eDNA analysis. Cervid eDNA (Appendix Table B) was found in one control root sample out of six tested (16%). A variety of DNA sequences (sciurid, cervid, hominid, and unmatchable) was detected in eleven of twenty two damaged root samples (50%). Three taxa were detected in the damaged roots, and their eDNA has a >90% match to known DNA sequences: Cervidae (deer family of mammals), Sciuridae (squirrel family of mammals), and Hominidae (human DNA resulting from contamination during sampling).

Soil eDNA analysis. Cervid eDNA was found in two soil tailings samples out of 24 tested (8.3%). A variety of DNA sequences was detected in ten of 24 soil tailings samples (41.7%). Three matchable taxa were detected in soil tailings, and these eDNA sequences had a >90% match to known DNA sequences: Cervidae (deer family of mammals), Sciuridae (squirrel family of mammals), and Hominidae (human DNA resulting from contamination during sampling).

With 825 identifiable reads in both soil and root samples, the combination of cervid (471 reads) and sciurid (354 reads) DNA sequences far outnumbered human DNA (19 total reads). Note that read-number cannot be perfectly correlated with species abundance in a sample because the DNA from each species interacts with the sequencing process in a slightly different way. Therefore, these data should be considered as providing a measure of presence or absence, not abundance.

The analysis revealed three genetic signatures of a rodent species belonging to tribe Marmotini, a taxon that includes antelope ground squirrels (*Ammospermophilus*), prairie dogs (*Cynomys*), marmots (*Marmotus*), Chinese rock squirrels (*Sciurotamias*), ground squirrels and rock squirrels (*Spermophilus*), and chipmunks (*Tamias*).

The genetic signatures range from 96.96 to 99.75% match ($e=0.0$) with the banked DNA sequence for Harris' antelope ground squirrel (*Ammospermophilus harrisi*) in the GenBank database maintained by the National Institutes of Health. The e-value is the number of expected

DNA sequences of similar match quality that could be found just by chance. The closer “e” is to zero, the better the quality of the match. This does not mean that the DNA came from *A. harrisii*. The DNA most likely originated from the locally abundant white-tailed antelope ground squirrel (*A. leucurus*), which may not have a 12S DNA barcode banked in the GenBank or BOLD databases. These two antelope ground squirrels are expected to have extremely similar DNA sequences because they belong to the same genus.

Scat eDNA analysis. The above same *Ammospermophilus* genetic signatures were also found in soil samples and in the rodent pellets found within Tiehm’s buckwheat subpopulation 2. The rodent pellets were also tested for plant eDNA. Twenty exact sequence variants (ESV) were detected in the rodent scat sample (Table 2). ESV represent unique combinations of DNA that are used to interrogate taxonomic identity, and in this scat sample the percent sequence match between ESV and known families ranged from 92-100% (Table 2). One ESV with a 100% match for the buckwheat genus, *Eriogonum* was present in the scat. A DNA barcode for Tiehm’s buckwheat does not yet exist in GenBank or BOLD, which means that the nearest genetic match is *Eriogonum crosbyae* with which it has a 100% sequence match and e-score that is indicative of a quality match ($e=2 \times 10^{-65}$).

Table 2. Exact sequence variants (ESV) derived from rodent scat obtained at Tiehm’s buckwheat (*Eriogonum tiehmii*) subpopulation 2. The identity of each ESV, the family or genus to which it best matches, the percent sequence match, and the number of reads (a rough, but not completely accurate measure of abundance) are provided. Percent match is the % of base pairs in the queried sequence that match to the base pairs of each species in the sequence library developed by Jonah Ventures.

ESV	Family/Genus	Percent Match	# Reads
ESV_009265	Caryophyllaceae	92	3293
ESV_051796		92	1324
ESV_098672		93	321
ESV_098812		92	124
ESV_099519		92	71
ESV_125549		93	25
ESV_100194		93	16
ESV_111660		92	11
ESV_100047		93	10
ESV_000156	Chenopodiaceae	100	837
ESV_074395		94	429
ESV_098572		95	174
ESV_098772		93	154
ESV_099012		97	98
ESV_060459		99	73
ESV_111461		95	45
ESV_000023		100	38
ESV_000143	Malvaceae	100	96
ESV_000189	Poaceae	100	25
ESV_000021	Polygonaceae: <i>Eriogonum</i>	100	111

No sciurid (squirrel family) eDNA was found in the control samples. These data strongly support the hypothesis that a diurnal rodent in the genus *Ammospermophilus* was responsible for damage to the Tiehm's buckwheat population at Rhyolite Ridge in the late summer and early fall of 2020. The genetic analysis is supported by morphological evidence consisting of rodent incisor marks on roots of damaged plants found in subpopulations 1, 2, 4, and 6 (Figure 1).



Depredated root that shows clear incisor marks along its length.



Typical damage found in cut plants that were found laying on the soil.



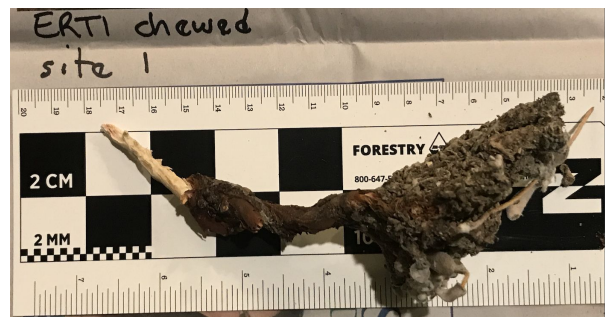
Plant showing damaged, but intact roots still connecting plant to substrate in a way unlikely to have been achieved by hominid tool use.



Close up view of plant pictured directly above. Roots are clearly damaged almost 360° around root, which supports rodent herbivory hypothesis.



A beautiful specimen of *E. tiehmii* in which two areas of depredation can be seen at the top of the photo near the notebook. In these two areas, the roots appeared to be excavated by a non-human vertebrate. The rest of the plant was intact and healthy. The appearance of this plant does not support the hominid poaching hypothesis.



Stripped roots (top left and right) from plants at subpopulation 1. By comparison note dark brown root covering still intact on control plant (lower right) that was excavated by humans during sample collection on 10/2/20.



Stripped roots on plants from subpopulation 2.



Stripped roots on plants from subpopulation 4.

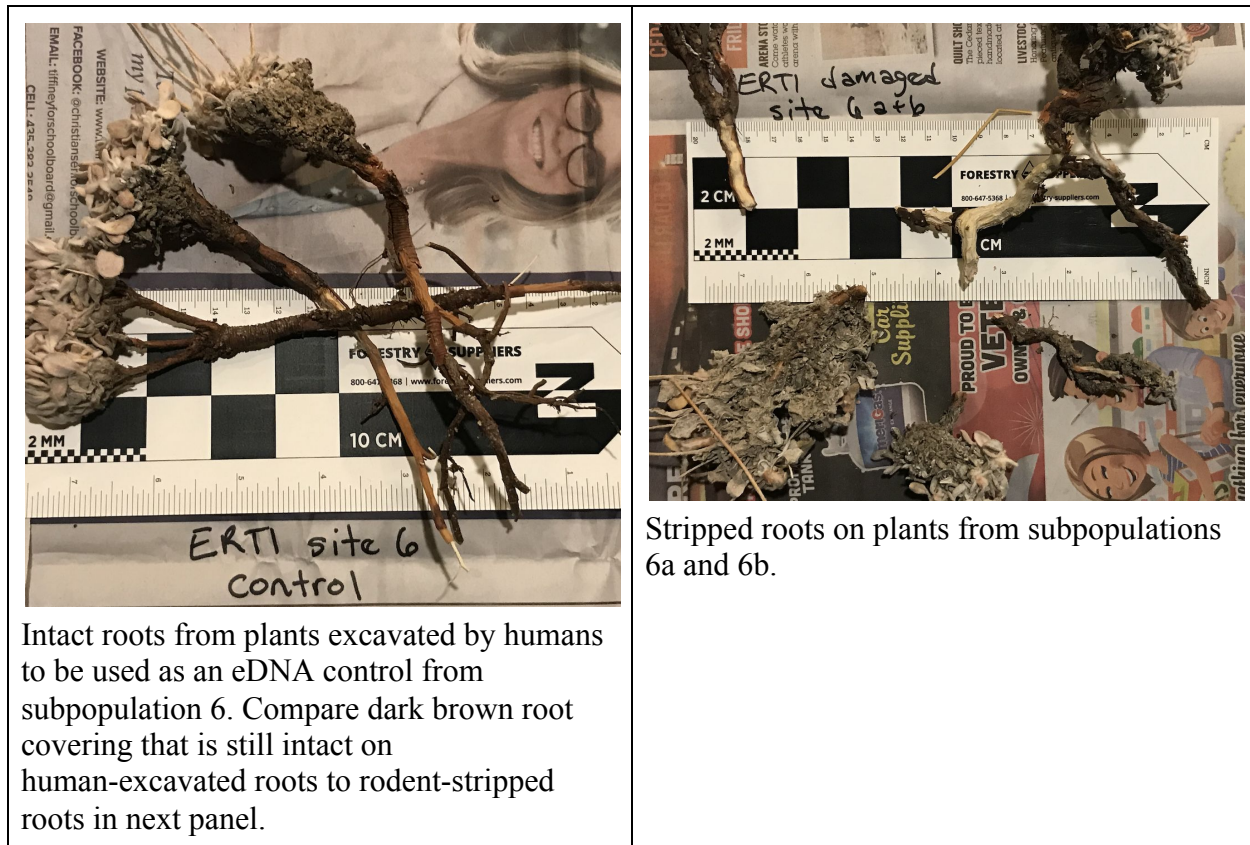


Figure 1. Photographs of depredated roots and control (untouched) roots used for genetic analysis in this study.

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END OF REPORT. APPENDIX WITH TWO TABLES FOLLOWS.

APPENDIX

Table A. Samples tested for presence of eDNA from herbivores, identification of herbivore DNA from scat, and presence of *Eriogonum* DNA in scat collected from Tiehm’s buckwheat subpopulation 2.

Subpop- ulation	Sample Type	Description of sample	Pooled sample info	DNA barcode:
1	control	roots from 1 untouched plant in 10X TE buffer	NA	vertebrates
1	control	roots from 1 untouched plant in 10X TE buffer	NA	vertebrates
1	control	roots from 1 untouched plant in 10X TE buffer	NA	vertebrates
1	roots	mixed damaged roots from 1-3 plants in 1X TE buffer	1-3 plants	vertebrates
1	roots	mixed damaged roots from 1-3 plants in 1X TE buffer	1-3 plants	vertebrates
1	roots	mixed damaged roots from 1-3 plants in 1X TE buffer	1-3 plants	vertebrates
1	roots	mixed damaged roots from 1-3 plants in 1X TE buffer	1-3 plants	vertebrates
1	roots	mixed damaged roots from 1-3 plants in 1X TE buffer	1-3 plants	vertebrates
2	roots	mixed damaged roots from 1-3 plants in 1X TE buffer	1-3 plants	vertebrates
2	roots	mixed damaged roots from 1-3 plants in 1X TE buffer	1-3 plants	vertebrates
2	roots	mixed damaged roots from 1-3 plants in 1X TE buffer	1-3 plants	vertebrates
2	roots	mixed damaged roots from 1-3 plants in 1X TE buffer	1-3 plants	vertebrates
2	roots	mixed damaged roots from 1-3 plants in 1X TE buffer	1-3 plants	vertebrates
2	roots	mixed damaged roots from 1-3 plants in 1X TE buffer	1-3 plants	vertebrates
4	roots	mixed damaged roots from 1-3 plants in 1X TE buffer	1-3 plants	vertebrates
4	roots	mixed damaged roots from 1-3 plants in 1X TE buffer	1-3 plants	vertebrates
4	roots	mixed damaged roots from 1-3 plants in 1X TE buffer	1-3 plants	vertebrates
4	roots	mixed damaged roots from 1-3 plants in 1X TE buffer	1-3 plants	vertebrates
4	roots	mixed damaged roots from 1-3 plants in 1X TE buffer	1-3 plants	vertebrates
4	roots	mixed damaged roots from 1-3 plants in 1X TE buffer	1-3 plants	vertebrates

6	control	roots from 1 untouched plant in 10X TE buffer	NA	vertebrates
6	control	roots from 1 untouched plant in 10X TE buffer	NA	vertebrates
6	control	roots from 1 untouched plant in 10X TE buffer	NA	vertebrates
6	roots	mixed damaged roots from 1-3 plants in 1X TE buffer	1-3 plants	vertebrates
6	roots	mixed damaged roots from 1-3 plants in 1X TE buffer	1-3 plants	vertebrates
6	roots	mixed damaged roots from 1-3 plants in 1X TE buffer	1-3 plants	vertebrates
6	roots	mixed damaged roots from 1-3 plants in 1X TE buffer	1-3 plants	vertebrates
6	roots	mixed damaged roots from 1-3 plants in 1X TE buffer	1-3 plants	vertebrates
1	soil	soil from 4 control sites with undisturbed soil	controls 1-4	vertebrates
1	soil	Soil tailings from within 30 cm radius of damaged plant	1-4	vertebrates
1	soil	Soil tailings from within 30 cm radius of damaged plant	5-8	vertebrates
1	soil	Soil tailings from within 30 cm radius of damaged plant	9-11	vertebrates
1	soil	Soil tailings from within 30 cm radius of damaged plant	12-14	vertebrates
1	soil	Soil tailings from within 30 cm radius of damaged plant	15-17	vertebrates
1	soil	Soil tailings from within 30 cm radius of damaged plant	18-20	vertebrates
2	soil	soil from 4 control sites with undisturbed soil	controls 1-4	vertebrates
2	soil	Soil tailings from within 30 cm radius of damaged plant	1-3	vertebrates
2	soil	Soil tailings from within 30 cm radius of damaged plant	4-6	vertebrates
2	soil	Soil tailings from within 30 cm radius of damaged plant	7-9	vertebrates
2	soil	Soil tailings from within 30 cm radius of damaged plant	10-11	vertebrates
2	soil	Soil tailings from within 30 cm radius of damaged plant	12-13	vertebrates
2	soil	Soil tailings from within 30 cm radius of damaged plant	14-15	vertebrates
4	soil	soil from 4 control sites with undisturbed soil	controls 1-4	vertebrates
4	soil	Soil tailings from within 30 cm radius of damaged plant	1-3	vertebrates
4	soil	Soil tailings from within 30 cm radius of damaged plant	4-6	vertebrates
4	soil	Soil tailings from within 30 cm radius of damaged plant	7-9	vertebrates
4	soil	Soil tailings from within 30 cm radius of damaged plant	10-11	vertebrates
4	soil	Soil tailings from within 30 cm radius of damaged plant	12-13	vertebrates
4	soil	Soil tailings from within 30 cm radius of damaged plant	14-15	vertebrates
6	soil	soil from 4 control sites with undisturbed soil	controls 1-4	vertebrates
6a	soil	Soil tailings from within 30 cm radius of damaged plant	S1, 1-4	vertebrates
6a	soil	Soil tailings from within 30 cm radius of damaged plant	S1, 5-8	vertebrates
6a	soil	Soil tailings from within 30 cm radius of damaged plant	S1, 9-11	vertebrates
6a	soil	Soil tailings from within 30 cm radius of damaged plant	S1, 12-14	vertebrates
6b	soil	Soil tailings from within 30 cm radius of damaged plant	S1, 15-17	vertebrates

6b	soil	Soil tailings from within 30 cm radius of damaged plant	S1, 18-20	vertebrates
2	scat	6 rodent pellets to examine for vertebrate DNA	NA	vertebrates
2	scat	6 rodent pellets to examine for presence of buckwheat DNA	NA	plants

Table B. DNA barcode sequence data and most closely matching taxon for all samples in which >10 sequence reads were present. Matches between the sample DNA and database DNA were at least 90% similar. A single sample may contain more than one genetic sequence from more than one taxon. Unknown DNA sequences usually can be attributed to DNA sequences that contain shared, common sequence information that make them indistinguishable between taxa. The number of reads is the number of DNA sequences that were detected in the sample.

Sample ID	Subpopulation	Type	DNA barcode sequence	Taxon/# reads
26055	1	control	Available upon request	Unknown/ 13
26056	1	control	none	none
26057	1	control	none	none
26058	1	roots	none	none
26059	1	roots	CTTAGCCCTAAACATAAACATCCAATAAACAGAATGTTCCGCCAGAG TACTACTAGCAACGGCCTAAAACTCAAAGGACTTGGCGGTGCTTTAC ATCCCTCTAGAGGAGCCTGTTCTATAATCGATAAACCCCGATATACCT CACCACCTTTAGCAATTATCAGCCTATATACCGCCATCTTCAGCAAAC CCTAATAAGGTCTAACAGTAAGCAGGATAATTATACATTAATACGTT AGGTCAAGGTGTAGCCTATAAGGTGGAAAGAAATGGGCTACATTTTC TATTTTTCAGAATAAAATACTCAGGATAGCTTTTATGAAACTTAAAGCA TAAGGCGGATTAGTAGTAAGTTAAGAATAGAGAGCTTAACTGAATC GGGCAATAAAGCACGCAC	Sciuridae/ 50
26059	1	roots	Available upon request	Unknown/ 67
26059	1	roots	Available upon request	Unknown/ 13
26060	1	roots	Available upon request	Unknown/ 77
26060	1	roots	CTTAGCCCTAAACACAAATAATTACATCAACAAAATTATTCGCCAGA GTACTACCGGCAATAGCTTAAAACTCAAAGGACTTGGCGGTGCTTTA TACCTTTCTAGAGGAGCCTGTTCTATAATCGATAAACCCCGATAAACC TTACCACCCCTTGCTAATTCAGTCTATATACCACCATCTTCAGCAAAC CCTAAAAAAGGAATAAAAGTAAGCTCAATCATATTACATAAAAAACGT TAGGTCAAGGTGTAACCTATGGGATGGAAAGAAATGGGCTACATTTT CTAACTTAAGAAAATCCATTATGAAAATTATTATGAAATTAATAATTA AAGGAGGATTAGCAGTAAACTAAGAATAGAGTGCTTAGTTGAATTA GGCCATGAAGCACGCAC	Cervidae/ 27
26061	1	roots	CTTAGCCCTAAACATAAACACTCAATAAACAGAATGTTCCGCCAGAA TACTACTAGCAATGGCCTAAAACTCAAAGGACTTGGCGTTGCTTTACA TCCCTCTAGAGGAGCCTGTTCTATAATCGATAAACCCCGATACATCTC ACCACCTTTAGCAATTATCAGCCTATATACCGCCATCTTCAGCAAACC CTAATAAGGTCTAACAGTAAGCAGGATAATTTACATTAATACGTTA GGTCAAGGTGTAGCCTATAAGGTAGGAAGAAATGGGCTACATTTTCT ATTTTTCAGAATAAAATACTCAGGATAGCTTTTATGAACTTAAAGCAT AAGGCGGATTAGTAGTAAGTTAAGAATAGAGAGCTTAACTGAATTG GGCAATAAAGCACGCAC	Sciuridae/ 58
26062	1	roots	Available upon request	Unknown/ 16
26063	2	none	none	none
26064	2	roots	none	none
26065	2	roots	none	none
26066	2	roots	none	none

26067	2	roots	Available upon request	Unknown/ 118
26068	2	roots	none	none
26069	4	roots	none	none
26070	4	roots	none	none
26071	4	roots	none	none
26072	4	roots	none	none
26073	4	roots	CTTAGCCCTAAACACAAATAATTATATCAACAAAATTATTCGCCAGA GTACTACCGGCAATAGCCTAAAACTCAAAGGACTTGGCGGTGCTTTA TACCCTTCTAGAGGAGCCTGTTCTATAATCGATAAACCCCGATAAACC TTACCACCCCTTGCTAATTCAGTCTATATACCGCCATCTTCAGCAAAC CCTAAAAAGGAATAAAAGTAAGCTTAATCATTTTACATAAAAAACGTT AGGTCAAGGTGTAACCTATGGGGTGGAAAGAAATGGGGTACATTTTC TAACTTAAGAAAATCTATCACGAAAATTATTATGAAAATTAATAATT AAAGGAGGATTTAGCAGTAACTAAGAATAGAGTGCTTAGTTGAATT AGGCCATGAAGCACGCAC	Cervidae/ 130
26074	4	roots	Available upon request	Unknown/ 13
26074	4	roots	Available upon request	Unknown/ 15
26075	6	control	none	none
26076	6	control	CTTAGCCCTAAACACAAATAATTATATCAACAAAATTATTCGCCAGA GTACTACCGGCAATAGCCTAAAACTCAAAGGACTTGGCGGTGCTTTA TACCCTTCTAGAGGAGCCTGTTCTATAATCGATAAACCCCGATAAACC TTACCACCCCTTGCTAATTCAGTCTATATACCGCCATCTTCAGCAAAC CCTAAAAAGGAATAAAAGTAAGCTTAATCATTTTACATAAAAAACGTT AGGTCAAGGTGTAACCTATGGGGTGGAAAGAAATGGGGTACATTTTC TAACTTAAGAAAATCTATCACGAAAATTATTATGAAAATTAATAATT AAAGGAGGATTTAGCAGTAACTAAGAATAGAGTGCTTAGTTGAATT AGGCCATGAAGCACGCAC	Cervidae/ 263
26077	6	control	none	none
26078	6	roots	CTTAACCCTAAACTCGAATAGTTAGATCAACAAAATTGTTCAACAGA ACACTACAAGCAACAGCTTAAAACTCAAAGGACTTGGCGGTGCTTTA TATCCCTCTAAAGGAGCCTGTTCTATAATCGATAAACCCCAATTTACC TCACCACCTCTTGCCCAGCCTATATACCTCCATCTTCAGCAAACCTG GAAAGGCCACAGAGTAAGCACAAGTATCTACATAAAAAACGTTAGGTC AAGGTGTAGCCCATGAGGTGGCAAGAAATAGGTATGTTTTCTACATC CAGAAAAATCTCGCGACAACCGTTATGAAATCTAAGGGCTCAAGGAG GATTTAGCAATAAATTGAGAGCAGAGTGTTAATCGAATAAGGCCAT GAAGCACGCAC	Human/ 11
26079	6	roots	none	none
26080	6	roots	none	none
26081	6	roots	none	none
26082	6	roots	none	none
26083	1	Soil control	none	none
26084	1	Soil tailings	none	none
26085	1	Soil	CTTAGCCCTAAACACAAATAATTATATCAACAAAATTATTCGCCAGA	Cervidae/ 35

		tailings	GTACTACCGGCAATAGCCTAAAACTCAAAGGACTTGGCGGTGCTTTA TACCCTTCTAGAGGAGCCTGTTCTATAATCGATAAACCCCGATAAAC TTACCACCCCTTGCTAATTCAGTCTATATACCGCCATCTTCAGCAAAC CCTAAAAAGGAATAAAAGTAAGCTTAATCATTTTACATAAAAAACGTT AGGTCAAGGTGTAACCTATGGGGTGGAAAGAAATGGGCTACATTTTC TAACTTAAGAAAATCTATCACGAAAATTATTATGAAAATTAATAATT AAAGGAGGATTTAGCAGTAACTAAGAATAGAGTGCTTAGTTGAATT AGGCCATGAAGCACGCAC	
26086	1	Soil tailings	Available upon request	Unknown/ 15
26087	1	Soil tailings	Available upon request	Unknown/ 46
26088	1	Soil tailings	CTTAGCCCTAAACACAAATAATTATATCAACAAAATTATTCGCCAGA GTACTACCGGCAATAGCCTAAAACTCAAAGGACTTGGCGGTGCTTTA TACCCTTCTAGAGGAGCCTGTTCTATAATCGATAAACCCCGATAAAC TTACCACCCCTTGCTAATTCAGTCTATATACCGCCATCTTCAGCAAAC CCTAAAAAGGAATAAAAGTAAGCTTAATCATTTTACATAAAAAACGTT AGGTCAAGGTGTAACCTATGGGGTGGAAAGAAATGGGCTACATTTTC TAACTTAAGAAAATCTATCACGAAAATTATTATGAAAATTAATAATT AAAGGAGGATTTAGCAGTAACTAAGAATAGAGTGCTTAGTTGAATT AGGCCATGAAGCACGCAC	Cervidae/ 16
26088	1	Soil tailings	CTTAGCCCTAAACACAAATAATTACACAAAACAAAATTGTTGCCAGA GTACTACTAGCGGAACAGCTTAAAACTCAAAGGACTTGGCGGTGCT TTATACCCTTCTAGAGGAGCCTGTTCTATAATCGATGAACGCTGATAA ACCGCAGCAGTCCTTGCTAATACAGTCTATACACCACCATCTTCAGCA AACCTGAAAAGAAACAAAAGTAAGCACAAATCATAGTACATAAAAA CATTAGGTCAAGGTGTAACCTATGGAGTGGGAAGAAATGCACTACAT CTTCTAATTTAAGAAAACCAATACGGAAGTTACTATGAACTAATA ACCAAAGGAGGATTTAGTAGTAACTAAGAATAGAGTGCTTAGTTGA ACTAGGCCATGAAGCATGCAC	Cervidae/ 34
26089	1	Soil tailings	none	none
26090	2	Soil control	Available upon request	Unknown/ 13
26091	2	Soil tailings	none	none
26092	2	Soil tailings	none	none
26093	2	Soil tailings	none	none
26094	2	Soil tailings	none	none
26095	2	Soil tailings	Available upon request	Unknown/ 23
26095	2	Soil tailings	Available upon request	Unknown/ 19
26096	2	Soil tailings	none	none

26097	4	Soil control	Available upon request	Unknown/ 98
26098	4	Soil tailings	none	none
26099	4	Soil tailings	none	none
26100	4	Soil tailings	Available upon request	Unknown/ 14
26100	4	Soil tailings	Available upon request	Unknown/ 22
26101	4	Soil tailings	Available upon request	Unknown/ 14
26102	4	Soil tailings	Available upon request	Unknown/ 25
26102	4	Soil tailings	Available upon request	Unknown/ 16
26102	4	Soil tailings	Available upon request	Unknown/ 11
26103	4	Soil tailings	none	none
26104	6	Soil control	none	none
26105	6a	Soil tailings	none	none
26106	6a	Soil tailings	none	none
26107	6a	Soil tailings	none	none
26108	6a	Soil tailings	none	none
26109	6b	Soil tailings	CTTAGCCCTAAACATAAAACATCCAATAAAACAAGAATGTTCCGCCAGAG TACTACTAGCAACGGCCTAAAACCTCAAAGGACTTGGCGGTGCTTTAC ATCCCTCTAGAGGAGCCTGTTCTATAATCGATAAACCCCGATATACCT CACCACCTTAGCAATTATCAGCCTATATACCGCCATCTTCAGCAAAC CCTAATAAGGTCTAACAGTAAGCAGGATAATTATACATTAATACGTT AGGTCAAGGTGTAGCCTATAAGGTGGAAGAAATGGGCTACATTTTC TATTTTTCAGAATAAAATACTCACGATAGCTTTTATGAAACTTAAAGCA TAAGGCGGATTAGTAGTAAGTTAAGAATAGAGAGCTTAACCTGAATC GGGCAATAAAGCACGCAC	Sciuridae/ 69
26109	6b	Soil tailings	Available upon request	Unknown/ 15
26110	6b	Soil tailings	CTTAGCCCTAAACATAAAACATCCAATAAAACAAGAATGTTCCGCCAGAG TACTACTAGCAACGGCCTAAAACCTCAAAGGACTTGGCGGTGCTTTAC ATCCCTCTAGAGGAGCCTGTTCTATAATCGATAAACCCCGATATACCT	Sciuridae/ 21

			CACCACCTTTAGCAATTATCAGCCTATATACCGCCATCTTCAGCAAAC CCTAATAAGGTCTAACAGTAAGCAGGATAATTATACATTAATACGTT AGGTCAAGGTGTAGCCTATAAGGTGGAAAGAAATGGGCTACATTTTC TATTTTTCAGAATAAAATACTCACGATAGCTTTTATGAAACTTAAAGCA TAAGGCGGATTTAGTAGTAAGTTAAGAATAGAGAGCTTAACTGAATC GGGCAATAAAGCACGCAC	
26111	2	rodent pellets	CTTAGCCCTAAACATAAACATCCAATAAACAAAGAATGTTGCCAGAG TACTACTAGCAACGGCCTAAAACCTCAAAGGACTTGGCGGTGCTTTAC ATCCCTCTAGAGGAGCCTGTTCTATAATCGATAAAACCCCGATATACCT CACCACCTTTAGCAATTATCAGCCTATATACCGCCATCTTCAGCAAAC CCTAATAAGGTCTAACAGTAAGCAGGATAATTATACATTAATACGTT AGGTCAAGGTGTAGCCTATAAGGTGGAAAGAAATGGGCTACATTTTC TATTTTTCAGAATAAAATACTCACGATAGCTTTTATGAAACTTAAAGCA TAAGGCGGATTTAGTAGTAAGTTAAGAATAGAGAGCTTAACTGAATC GGGCAATAAAGCACGCAC	Sciuridae/ 133
26111	2	rodent pellets	CTTAGCCCTAAACATAAACATCCAATAAACAAAGAATGTTGCCAGAG TACTACTAGCAACGGCCTAAAACCTCAAAGGACTTGGCGGTGCTTTAC ATCCCTCTAGAGGAGCCTGTTCTATAATCGATAAAACCCCGATATACCT CACCACCTTTAGCAATTATCAGCCTATATACCGCCATCTTCAGCAAAC CCTAATAAGGTCTAACAGTAAGCAGGATAATTATACATTAATACGTT AGGTCAAGGTGTAGCCTATAAGGTGGAAAGAAATGGGCTACATTTTC TATTTTTCAGAATAAAATACTCACGATAGCTTTTATGAAACTTAAAGCA TAAGGCGGATTTAGTAGTAAGTTAAGAATAGAGAGCTTAACTGAATT GGGCAATAAAGCACGCAC	Sciuridae/ 14
26112	2	rodent pellets	GACTTAATTGGTTGAGCCTTAGTATGGAAACCTACTAAGTGAGAACTT TCAAAATCAGAGAAACCTGGAATTAATAAAAAATGGGCAATCCTGAG CCAACCTCTGCTTTCCAAACTTTCCAAAAAGGAGAATAAAAAAAG	<i>Eriogonum</i> / 111
26112	2	rodent pellets	Plant eDNA from the following families were also found in the rodent pellets: Caryophyllaceae, Chenopodiaceae, Malvaceae, and Poaceae. Sequence data available upon request. Read numbers, ESV, and percent match in Table 2.	

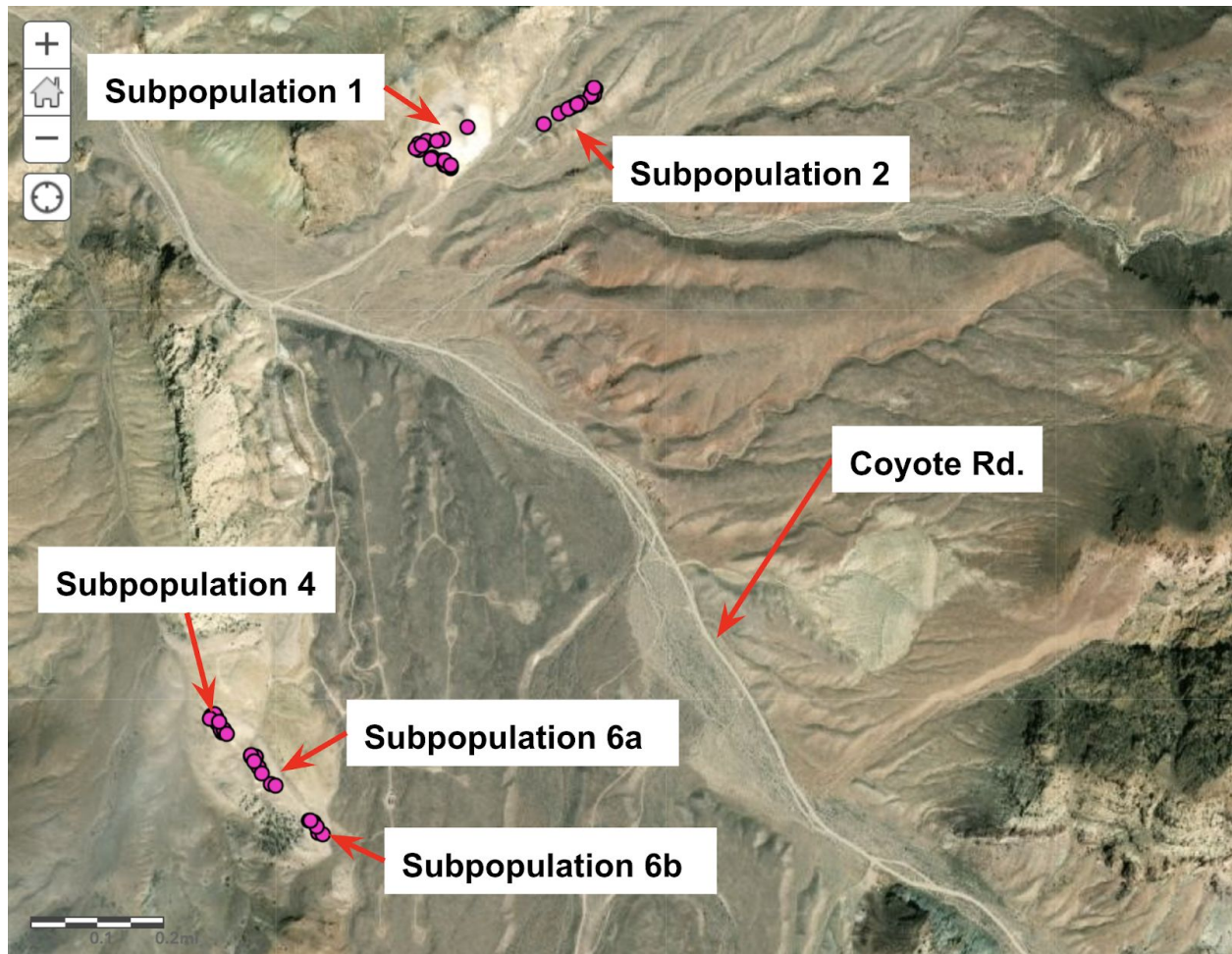


Figure A. eDNA collection sites at Tiehm's buckwheat (*Eriogonum tiehmii*) subpopulations 1, 2, 4, 6a and 6b. Pink dots indicate where soil samples and roots were collected on 10/2/20.